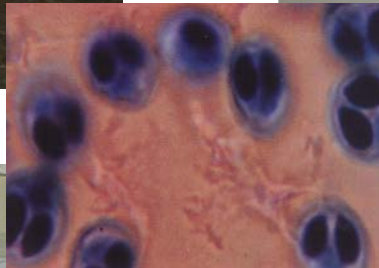


National Wild Fish Health Survey

Laboratory Procedures Manual



U.S. Fish & Wildlife Service

Division of Fish Hatcheries
Washington, D.C.

Second Edition - June 2004

This publication was originally produced in 2000 for the U.S. Fish and Wildlife Service, to describe the protocols and procedures utilized in the National Wild Fish Health Survey (NWFHS). The second edition was completed in June 2004 to include new PCR methodologies for viral testing. The NWFHS Laboratory Procedures Manual is intended for use by USFWS Fish Health Centers, but may also be helpful to fishery professionals, the technically oriented fishery industry, and students of biological or fishery sciences.

This Manual is accessible via the internet at <http://www.r1.fws.gov/canvfhc/nwfhsman.htm> . CD-ROM copies may be requested from the U.S. Fish and Wildlife Service at the address on the title page. All recipients are requested to provide feedback on their use of this Manual and offer suggestions for improvements. The material may be updated annually if significant changes are made in the detection or corroborative testing methods, or in the overall Survey procedures.

To cite this publication:

True, K. (Ed) 2004. National Wild Fish Health Survey – Laboratory Procedures Manual. Second Edition. U.S. Fish and Wildlife Service, Anderson, CA.

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PREFACE

There are many published sources for laboratory procedures used in the diagnosis of finfish diseases. The National Wild Fish Health Survey (NWFHS) – Laboratory Procedure Manual is not intended to be comprehensive in its treatment of this large subject area. Many of the major fish diseases that occur within the United States and throughout the world have not been documented in wild fish populations. Consequently, the NWFHS (Survey) has targeted major fish pathogens that are considered a risk to both natural and artificially produced fish populations. The NWFHS Laboratory Procedure Manual (Manual) contains protocols for these major pathogens, and also provides a general scheme of approach to allow detection of new or exotic agents through the disciplines of virology, bacteriology, parasitology and histology. Pathogens of Regional Importance (PRI) are included for informational purposes; however specific protocols have not been included, in most cases, for these pathogens.

The Survey includes methodologies that provide the highest sensitivity for detection of target fish pathogens in subclinical wild fish populations. For this reason, Polymerase Chain Reaction¹ (PCR) technology is included in the Survey as an additional tool to corroborate detection of specific diseases (Whirling Disease, Bacterial Kidney Disease, Ceratomyxosis, and viral agents such as IHNV and VHSV). In this application of PCR as a “corroborative test method”, it is not the intent of the Survey to imply that PCR is to replace “gold standards” for detecting or confirming specific fish pathogens. Rather, PCR is being utilized as a secondary detection tool to corroborate the presence of a pathogen that has been detected by standard methods. The intent of the Survey is to test the PCR technique along side standard detection methods such as Pepsin-Trypsin Digest, ELISA, and Virology. This PCR data will add to our knowledge of both fish diseases and the performance of molecular tools as detection methodologies. Traditional confirmation tests such as histology (for Whirling Disease and ceratomyxosis), and serum neutralization for viral agents are also included for the Survey.

The foremost purpose of this Manual is to provide a working document of very detailed information for the U.S. Fish and Wildlife Service - Fish Health Center staff and clients regarding the daily procedures in which we conduct the Wild Fish Health Survey. Where appropriate, the methods herein follow those described in Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens, 2004, 6th edition. American Fisheries Society, Fish Health Section, Bethesda, Maryland, commonly referred to as the AFS-FHS Blue Book (2004). Additional protocols are included for the Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) techniques. In several instances protocols cited from other investigators in the published literature have been referenced as well. As with most laboratory manuals, this document will be updated as new and improved procedures become available.

NOTE: Mention of brand names or trademarks, or any specific equipment in the text of this Manual is not an endorsement of any particular product by the U.S. government or the Fish and Wildlife Service. The products mentioned serve only as descriptive models for the reader, any comparable product can be used at the discretion of the reader.

¹The PCR Process is covered by Patents owned by Hoffman-LaRoche, Inc.

EDITOR

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ACKNOWLEDGMENTS

This Manual would not be possible without the generous contribution by Theodore R. Meyers of the Alaska Department of Fish and Game. The first edition of the Manual was based in large part on the Alaska Department of Fish and Game, Fish Pathology Section Laboratory Manual (Meyers, 1997). We have edited, updated and adapted their manual to reflect the needs of the Wild Fish Health Survey. The USFWS recognizes and thanks the efforts of Dr. Meyers, Jill Follett, Joan Thomas, Marie Fried, Sally Short, and Tamara Burton for their research and compilation of much of the information in the first edition.

Several other key individuals contributed to the basis of the information and protocols contained in this Manual. Of special note are Dr. James Winton of the Western Fisheries Research Center (USGS-BRD), Seattle, Washington for his technical review of this manuscript; Ron Pascho (USGS-BRD) for his development of the ELISA for detection of Bacterial Kidney Disease; Dr. Karl Andree and Dr. Ron P. Hedrick of the University of California (Davis) for the methodology for detecting Whirling Disease by Polymerase Chain Reaction (PCR); and many other dedicated researchers and technicians who continue to contribute to our knowledge of fish diseases.

A special acknowledgment is in order for Mary Ellen Mueller and William Knapp of the Division of Hatcheries for their support of the National Wild Fish Health Survey during its inception in 1996-1997.

And finally, recognition and appreciation to the fish health biologists from the nine fish health centers across the country. These individuals have worked diligently over the past several years to make the Survey successful by developing partnerships, collecting thousands of fish samples, performing the laboratory testing, and managing the information in the NWFHS Database. In addition to the work required for the Survey, each Fish Health Center contributed to the Second Edition by updating the written protocols that comprise this Manual.